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Cardioprotective effects of timosaponin B II from *Anemarrhenae asphodeloides* Bge on isoproterenol-induced myocardial infarction in rats

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ABSTRACT

The aim of the present study was to investigate the cardioprotective effects of Timosaponin B II (TB), a main bioactive constituent from *Anemarrhenae asphodeloides* Bge, on an isoproterenol (ISO)-induced myocardial infarction model in rats and explore its underlying mechanisms. Rats were treated with TB (50 mg/kg, 100 mg/kg) or diltiazem hydrochloride (DH, 5 mg/kg) by gastric gavage for five days. At the 4th and 5th days, myocardial injury was induced by ISO injection (85 mg/kg) at an interval of 24 h for 2 consecutive days. After the induction, rats were anaesthetized with pentobarbital sodium (30 mg/kg) to record the electrocardiogram. Our research showed that ISO administration resulted in significant elevations in the ST-segment, the levels of cardiac injury biomarkers creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH), and concentrations of serum proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Pretreatment with TB significantly reversed these alterations induced by ISO challenge. The cardioprotective effects of TB were further proved by the histopathological examination. Exploration of the underlying mechanisms of its actions revealed that TB pretreatment restored the ISO-induced decrease of super oxide dismutase (SOD) and the increase of malondialdehyde (MDA). Meanwhile, we found that the enhancement of antioxidant defense system might be associated with the increased heme oxygenase isoform 1 (HO-1) induction and activated nuclear respiratory factor 2 (Nrf-2) translocation. Furthermore, the present research also demonstrated that nuclear translocation of Nrf-2 and subsequent HO-1 expression might be associated with nuclear factor kappa B (NF-κB) pathway activation. Taken together, our finding demonstrated that TB might have a potential benefit in preventing ischemic heart diseases like myocardial infarction.

1. Introduction

Myocardial infarction refers to an acute condition of myocardium necrosis that caused by critical imbalance between the coronary blood supply and the demand of the myocardium [1]. Although great improvements have been made in medical care and clinical nursing, myocardial infarction still remains the leading cause of disability and mortality worldwide, even lasting to the year of 2020 [2–4]. Recent researches investigating the mechanisms that regulate myocardial infarction provide evidence that oxidative stress, induced by increased generation of reactive oxygen species, plays a critical role in the pathogenesis of myocardial infarction [5]. The increased oxygen stress damages the membrane lipids, carbohydrates, DNA and proteins, and thereby leads to cellular destruction, energy deficiency, and acceleration of cell death [5,6]. Thus, therapeutic intervention with antioxidant treatment may prevent these deleterious changes and attenuate myocardial dysfunctions induced by acute myocardial infarction [7].

Catecholamines overproduction induced by the adrenergic overstimulation is a great risk for cardiac dysfunction. It has been reported that supraphysiological levels of catecholamine in plasma

Abbreviations: TB, timosaponin B II; ISO, isoproterenol; DH, diltiazem hydrochloride; CK-MB, creatine kinase-MB; LDH, lactate dehydrogenase; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; SOD, super oxide dismutase; MDA, malondialdehyde; HO-1, heme oxygenase isoform 1; Nrf-2, nuclear respiratory factor 2; NF-κB, nuclear factor kappa B.

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may produce cardial dysfunction by affecting myocardial energy metabolism [8]. Isoproterenol (ISO), a β-adrenergic agonist and synthetic catecholamine, produces myocardial infarction at the sub-maximal dose as a non-invasive method in rodents [9]. Among the various mechanisms proposed for understanding the ISO-induced myocardial injury, large-scale generation of highly cytotoxic free radicals from oxidative metabolisms of catecholamines is commonly accepted. It is believed that free radicals reacting with proteins, lipids and nucleic acids cause lipid peroxidation, permeability alteration and integrity breakage of biological membrane, leading to irreversible damages to the function of myocardial membrane [10,11]. ISO-induced acute necrosis of myocardium presents increased releases of cardiac biomarker enzymes, abnormal changes of electrocardiograph and damaged cardiac functions [7,12]. Furthermore, pathophysiological alterations of ISO-induced acute myocardial necrosis in rodents, including the increased releases of cardiac biomarker enzymes, abnormal changes of electrocardiograph and damaged cardiac functions, are comparable with those occurred in human myocardial infarction [13,14], and thus widely adopted to study the cardioprotective efficacy of many drugs.

Phytomedicine has being used for the prevention and medication of cardiac ailments such as heart failure, coronary insufficiency and atherosclerosis from time immemorial. The rhizome of *Anemarrhenae asphodeloides* Bge, a traditional Chinese herbal drug, has long been included in Chinese traditional medical recipes for the treatment of various heart diseases [15,16]. TB, a main bioactive constituent in this herbal drug, has many pharmacological properties including antioxidation and anti-inflammation [17,18]. However, the effects of TB on isoproterenol induced myocardial infarction have remained unknown. In this study, the effects of TB pretreatment on ISO-induced myocardial infarction were measured and possible mechanisms were explored for this action.

2. Materials and methods

2.1. Reagents

TB (purity > 95%) was purchased from National Institutes for Food and Drug Control (Beijing, China). LDH, CK, SOD, MDA, IL-6 and TNF-α were determined by commercial kits produced by Nanjing KeyGEN Biotech. CO., LTD. (Nanjing, China) following the corresponding instructions. All antibodies were purchased from Cell Signaling Technology (Danvers, USA). Isoproterenol was purchased from Sigma–Aldrich Chemical Co., LLC., USA. Other reagents were of commercial analytical grade.

2.2. Ethics statement

All animal experiments were performed strictly in accordance with protocols approved by China Pharmaceutical University Medicine Animal Care and Use Committee.

2.3. Animals

50 Male SD rats (200–250 g, 8 weeks old) were purchased from the Animal Experiment Center of China Pharmaceutical University, China. The animals were maintained in a temperature-controlled room at 20–23 °C, with a relative humidity of 40–50% and a 12 h light/dark cycle. The rats had free access to food and water.

2.4. Induction of myocardial infarction and experimental design

Myocardial injuries in rats were induced by isoproterenol injection (Isoproterenol was dissolved in physiological saline and administered at the dose of 85 mg/kg body weight) subcutaneously at an interval of 24 h for 2 consecutive days as previously described [19]. After acclimatization for one week, the experimental animals were randomly divided into five groups of ten rats each: (1) Normal control group; (2) ISO group; (3) ISO + DH (5 mg/kg) group; (4) ISO + TB (50 mg/kg) group; and (5) ISO + TB (100 mg/kg) group. ISO + DH (5 mg/kg) group and ISO + TB (50 mg/kg, 100 mg/kg) groups were pretreated with drug (DH or TB) for five days, and subjected to ISO treatment at the 4th and 5th days.

After the final dose of ISO or other drugs, the rats were anesthetized with pentobarbital sodium anesthesia (30 mg/kg) and needle electrodes were inserted into the lead II position. Electrocardiograms (ECG) of the experimental animals were kept track of continually and ST-segment elevation or depression was also calculated. ST-segment alterations in the experimental rats were recorded under a BL-420S Biological Function Experiment System, Chengdu Thaimeng Technology Co. Ltd, (Chengdu, China).

Blood samples were collected from the retro-orbital plexus 24 h since the last ISO injection. After centrifugation at 4000 rpm for 10 min, serum was separated and stored at − 80 °C till biochemical analyses. Then the animals were sacrificed rapidly. The heart sample was removed and homogenized with ice cold phosphate buffer saline (50 mM, pH 7.4) in a tissue homogenizer. The obtained heart tissue homogenate (10% w/v) of different experimental animals was centrifuged at 12000 rpm for 45 min at 4 °C. The collected supernatant was used for the determination of following biochemical parameters. The content of tissue protein was tested by using Bradford reagent (Sigma–Aldrich) against bovine serum albumin (BSA) as standard.

2.5. Estimation of myocardial injury markers

The prepared serum samples were used for the determination of cardiac marker enzymes creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) using commercially available standard kits (Jiancheng Bioengineering Inc., China) by RT-9600 Semi-automatic Biochemical Analyzer. All measurements were performed according to the kits manufacturers' instructions.

2.6. Estimation of proinflammatory cytokines and antioxidant assay

The serum levels of IL-6, TNF-α were determined using an enzyme-linked immunosorbent assay (ELISA) kit (R&D System Inc, USA). SOD and MDA were assayed with commercially available standard kits (Jiancheng Bioengineering Inc., China) according to the manufacturer's instructions.

2.7. Western blot analysis

The heart tissue was chopped into pieces and homogenized in ice-cold RIPA buffer (0.1% phenylmethylsulfonyl fluoride), containing protease inhibitor (cOmplete Cocktail tablets, Roche Applied Science, Germany). The supernatant was collected after centrifugation at 12000 rpm for 20 min. The protein concentration was determined by using the BCA protein assay kit. Protein extracts were loaded and separated by an SDS-polyacrylamide gel electrophoresis, and the resolved proteins were transferred onto PVDF membranes. The membranes were blocked with 5% skim milk in Tris buffer saline and incubated at 4 °C overnight with separate primary antibodies, anti-Nrf-2 (abcam, 1:1000), anti–HO–1 (abcam, 1:2000), anti-p–NF–κB (CST, 1:1000), anti–NF–κB (CST, 1:1000), anti-p-IκBα (CST, 1:1000), anti-IκBα (CST, 1:1000), anti-p-IKKα (CST, 1:1000), anti-IKKα (CST, 1:1000), anti-IKKβ (CST, 1:1000) and anti-p-IKKβ (CST, 1:1000). After washing three times with Tris-